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Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation

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Abstract

Background and Aims The intrinsic nitrogen (N) supply capacity of soil is central to understanding the productivity of natural plant communities, and essential in the context of determining optimal fertilization rates for agricultural soils. However, it is largely unknown how nutrient availability affects plant mediated priming effects driving soil organic matter mineralisation and associated N-fluxes.

Methods We applied continuous, steady-state ^{13}C -CO₂ labelling to *Lolium perenne* grown in high and low productivity grassland soils to allow quantification of SOM- and root-derived soil CO₂ efflux. Nutrient treatments (N, P and K) were applied as repeated additions to soils, and impacts on source partitioned soil CO₂ efflux were assessed relative to unamended planted and fallow soils. Plants were clipped to uniform height at weekly intervals.

Results Increasing nutrient availability in both soils resulted in a reduction in plant-mediated SOM mineralisation and clipping of plants greatly lowered root-derived respiration but increased SOM mineralisation. Nutrient addition to fallow systems had no effect on SOM mineralisation in either soil. Plant growth stimulated SOM priming, concurrent mobilisation of N from SOM and subsequent plant N uptake in the high productivity soil. Priming was not observed in the low productivity soil due to its greater inherent organic matter stability, resulting in lowered plant-mediated and basal SOM mineralisation.

Conclusions That addition of nutrients reduced SOM mineralisation in planted systems but had no effect in fallow systems is indicative of nutrient availability specifically altering plant-mediated priming of SOM mineralisation. We suggest that plant-soil interactions mediating priming effects are an important determinant of productivity and that the magnitudes of these effects are modified by nutrient availability and soil-specific controls.

Introduction

Soil nitrogen (N) supply is limiting to plant productivity in the majority of temperate ecosystems (Vitousek and Howarth, 1991), and so intensive agriculture is dependent on large inputs of inorganic N fertiliser to maintain crop yields. The dominant form of N in soil is organic matter (typically > 99% of soil N, Weaver et al., 1994), with plant growth in unfertilised systems dependent on microbial mineralisation to sustain the much smaller, plant-available mineral N pools. This microbial-mediated flux of N from soil organic matter (SOM) to mineral forms is also quantitatively important for plant productivity in agricultural soils, even those receiving large inputs of fertiliser. For example, Engels and Kuhlmann, (1993) estimated that N mineralisation in unfertilised loamy soils under wheat cultivation averaged 119 kg N ha⁻¹ yr⁻¹, while McDonald et al. (2014) reported that SOM-N contributed 60% of barley biomass N when grown in a loamy soil receiving 120 kg N ha⁻¹ fertiliser N. Plant carbon (C) inputs to soil are thought to increase the soil's supply of N (Herman et al., 2006; Craine et al., 2007; Murphy et al., 2015; Rousk et al., 2016), however there is great uncertainty about the interactive effects of nutrient availability on plant-mediated SOM mineralisation and associated N-fluxes. Accounting for the intrinsic N supply capacities of soils is central to understanding the productivity of natural plant communities, and essential for determining optimal fertilisation rates for agricultural systems (Olfs et al., 2005; Dungait et al., 2012a).

The N supply from soil to plant can be defined as:

$$N_S = N_A + N_E + N_M - N_L \text{ (Eqn. 1)}$$

Where N_S is the total N supply potentially available for plant uptake, N_A is the standing pool of mineral N (available), N_E is the external supply of mineral N (from fertiliser or N-

deposition), N_M is the N mineralised from SOM (including plant residues and organic amendments), and N_L represents gaseous losses and leaching; but may also be considered to include N that is cycled from mineral to organic forms. Of these terms, N_A can be measured directly, and N_E is known for fertilisation inputs and can be estimated with reasonable certainty for N-deposition (Galloway, 1995). The remaining components, N_M and N_L , and the processes underlying them are highly variable across systems, both spatially and temporally (Schimel et al., 1989; Burke et al., 1997; Kieft et al., 1998). Further, although processes contributing to N_M and N_L are known, the magnitudes and relative contributions within interacting influences of root traits, soil, environment and management are poorly understood, limiting prediction of N availability for plant growth (Shepherd et al., 1996; Dungait et al., 2012a).

Across natural ecosystems plant adaptation to variation in N_S (largely determined by N_M) includes control of belowground allocation (root-to-shoot ratio), formation of mutualistic symbioses (mycorrhizal and N_2 -fixing), rooting strategies (e.g. depth and architecture) and plasticity in root development to exploit localised availability of nutrients (Dewar, 1993; Lynch, 1995; Drinkwater et al., 1998; Forde and Lorenzo, 2001; Smith and Read, 2008), resulting in recognised plant ecotypes adapted to their environment and competition/ co-existence within plant communities (Grubb, 1977; Johnson et al., 2010). These strategies are coupled with *in planta* mechanisms for nutrient use efficiency, such as luxury uptake (Chapin, 1980) and remobilisation of N from senescing tissues (Distelfeld et al., 2014) to support new growth. For agroecosystems, the nutrient saturation of soils through intensive fertilisation (N_E) reduces the reliance of plant productivity on microbial processes mediating N_M , meaning that plant production is effectively uncoupled from activities of the associated soil biota (Drinkwater and Snapp, 2007). This has important implications for N_L , because

whereas in unfertilised soils plant growth is tightly coupled to the flux of N into mineral pools (meaning that the size of these is constrained by plant uptake), fertiliser application saturates plant-available pools (at least for a period), resulting in potential for substantial gaseous and leaching losses (Vitousek et al., 1997).

The capacity of nutrient additions to uncouple plant-microbe interactions is recognised from the established inhibition of mycorrhizal and rhizobial symbioses under nutrient replete conditions (Johnson et al., 1997; Nanjareddy et al., 2014). However, it is also recognised that plant interactions with free-living components of the soil biota can substantially affect nutrient cycling rates in soil (Hamilton III and Frank, 2001; Paterson, 2003), but the effect of N application on these interactions is unknown.

Plant roots influence the physical and chemical soil environment (Hinsinger et al., 2003; Bronick and Lal, 2005; Chapman et al., 2012), but a key influence on microbial communities is the supply of organic substrates in rhizodeposition (Paterson et al., 2007). Associated changes in microbial activity and community structure have been demonstrated to alter rates of SOM mineralisation (priming effects), but this interactive effect has been difficult to quantify, meaning it is generally not considered in estimation of the contribution of N_M to plant productivity.

Given sufficient light and water, plants have an unlimited source of C through photosynthesis, but do not have direct access to the dominant stock of nutrients within soils (i.e. SOM). In contrast, soils are generally C-limiting environments for microbial communities (Schimel and Bennett, 2004; Brookes et al., 2009), but these communities (or components of them) have the physiological capacity to decompose SOM. That this potential for microbial communities to utilise SOM as substrate does not result in decomposer

proliferation to the point of complete depletion of SOM, indicates that in soil there are factors in soil limiting its use. Potential limiting factors, other than environmental conditions inhibiting microbial activity, are physical protection of organic matter within the soil matrix, complexation with soil minerals, chemical recalcitrance and availability of an alternative source of low molecular weight carbon, e.g. rhizodeposition (Lützow et al., 2006; Kleber, 2010). Recently, the importance of physicochemical mechanisms of stabilisation have been emphasised over more traditional concepts of SOM being composed of C-pools with differing rates of turnover, defined by their chemical composition (Dungait et al., 2012b). However, that priming effects do occur in a wide range of natural and managed soils (Kuzyakov, 2010), often substantially elevating rates of SOM decomposition (Cheng et al., 2014), would seem to suggest that there are C-pools that are accessible to microbial communities, but are only mineralised following input of labile C substrate. This interaction between C-input and mineralisation of native SOM has been interpreted as a microbial N-mining response, where microbial C-limitation is alleviated by the C input and increased microbial activity (enzyme production) results in mobilisation of limiting nutrients from SOM (Craine et al., 2007; Murphy et al., 2015). Implicit within this view is that SOM pools acted on by priming represent substrates that are not viable as C-sources for microbial growth, i.e. their recalcitrance requires greater use of C than is gained from their utilisation (Schimel and Bennett, 2004).

Consideration of priming as a N-mining response is supported by observations of altered patterns of microbial enzyme production (Sinsabaugh and Moorhead, 1994), and the finding that the primed flux from SOM is derived preferentially from N-rich components (Murphy et al., 2015). Consequently, plant-mediated SOM mineralisation represents a distinct component of N_M that may be subject to specific controls. This idea is supported by the

finding that rates of SOM mineralisation are insensitive to the abundance and composition of microbial communities (Kemmitt et al., 2008), except when labile C is present and priming is active (Garcia-Pausas and Paterson, 2011). If SOM pools acted on by priming are non-viable as C-substrates and are mineralised to mobilise N, it follows that N-mining responses may be down-regulated in the presence of abundant mineral N.

Here we investigated interactions between plant-mediated priming effects and the availability of mineral N for two soils contrasting in N_s and their capacities to support grassland productivity. We hypothesise that: 1) N-addition will reduce SOM mineralisation in planted soil, 2) that this will be a consequence of a reduced contribution from priming, and 3) that plant-mediated effects on N_M will be larger in the more productive soil, underpinning the capacity of this soil to support plant growth.

Materials and methods

Soil

Two grassland soils were chosen for this experiment: Grange, County Meath (6°40'07.597"W, 53°31'22.649"N) and Moorepark, County Cork (8°14'21.408"W, 52°10'15.187"N), Ireland. From herein the Grange and Moorepark soils are referred to as high and low productivity soil, respectively. The land management for these soils was grassland for greater than five years. These soils were previously characterised (McDonald et al., 2014) and were found to support contrasting grassland productivities through their respective N-supply capacities, but having very similar SOM contents (10 % and 8% for high and low productivity soil, respectively) and C-to-N (9 and 10 for the high and low productivity soil, respectively) ratios. Soils were sampled to 10 cm depth, sieved (2 mm) at field moisture content and stored at 4°C before use.

Steady-state labelling and experimental design

Soils were packed (1574 g dry weight) to a bulk density of 1 g cm⁻³ in 2 L pots. The dimensions of the pots were 110 mm x 110 mm x 200 mm (*l* x *b* x *h*). A PVC mesh (1 mm) was placed at the base of each pot to prevent soil loss. For each pot, a gas sampling chamber (250 mL headspace with gas inlet and outlet ports) was embedded to 2 cm into the soil. Four glass rods were inserted into the soil around the sampling chamber and held by an elastic band to ensure the chamber remained secure. For planted treatments, the perennial ryegrass seeds were sown outside the glass chamber ensuring that root and SOM-derived respiration was measured only.

Pots were placed in randomised blocks in a Perspex labelling chamber within a controlled environment room (Convion, Winnipeg, Canada) (Paterson et al., 2005; 2007). Soils were equilibrated for 14 d to the conditions applied in the experiment (see below). During this

period the pots were kept in the dark and maintained at 65% water holding capacity (WHC), by addition of deionised water every 2-3 days. On the day of sowing (Day 0) and throughout the remainder of the experiment, the labelling chamber was flushed with ^{13}C -depleted CO_2 at $385 \pm 7 \mu\text{mol mol}^{-1}$. This was achieved by blending CO_2 -free air (removed using a FT-IR Purge Gas Generator, Parker Balston model 75-62) and analytical grade CO_2 gas with a depleted $\delta^{13}\text{C}$ signature of -36.5‰ , using mass flow controllers (Flotech Solutions, Stockport, UK) and routed through a 20 L mixing tank (Paterson et al., 2005). The blended gas was supplied ($385 \pm 7 \mu\text{mol mol}^{-1} \text{CO}_2$) to the labelling chamber at a flow rate of 20 L min^{-1} using mass flow controllers (Flotech Solutions, Stockport, UK) in each gas-line. The CO_2 concentration in the exhaust from the labelling chamber was checked each day using an infrared gas analyser (EGM-4, PP-Systems, Amesbury, USA) and when excessive ($> 20 \mu\text{mol mol}^{-1}$) depletion of CO_2 in the labelling chamber occurred from photosynthesis, the CO_2 concentration of the inflow gas was increased. The controlled environment room maintained a day (14 h) and night (10 h) cycle. The light flux for the chamber was set at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level within the chamber. The temperature in the controlled environment room was altered to ensure a day temperature of 22.6°C was achieved in the labelling chamber and 18.0°C during the night. Watering and sampling of the plants was carried out during dark periods to reduce disturbance to the $\delta^{13}\text{C}$ signature of the CO_2 fixed by the plants. Plants were clipped to 3 cm above the soil surface, once a week from Day 21, clipped material was freeze-dried and weighed.

The experiment consisted of four treatments, applied to two soils: 1) unplanted, no nutrient addition 2) planted, no nutrient addition 3) planted with nutrient addition 4) unplanted with nutrient addition ($n=4$). The planted treatments were sown with *Lolium perenne* C.V. Kent to a density of 31.6 g m^{-2} . Nutrient treatments received combined NPK (4:1:2) additions twice

per week with watering to maintain 65% WHC, starting 14 days after sowing (total of 11 additions). Nitrogen was added as ammonium nitrate ($^{15}\text{NH}_4^{15}\text{NO}_3$, 5 atm%) at $0.018 \text{ mg N g}^{-1}$ soil per addition ($0.15 \text{ mg N g}^{-1}\text{soil}$ in total, equivalent to 130 kg N ha^{-1}), P was added as anhydrous sodium hydrogen phosphate, $0.002 \text{ mg P g}^{-1}\text{soil}$ per addition (0.04 mg P g^{-1} in total, $32.5 \text{ kg P ha}^{-1}$) and K was added as potassium sulphate at $0.004 \text{ mg N g}^{-1}$ per addition (0.07 mg K g^{-1} in total, 65 kg K ha^{-1}).

From 21 days after sowing, soil CO_2 flux rates and ^{13}C isotopic ratios were determined for all treatments. After CO_2 measurements and isotopic composition samples were taken, plants were clipped (3 cm from soil surface) and 24 h later another CO_2 collection for flux rate and isotopic composition was taken for all treatments. For each soil CO_2 efflux collection, the gas chambers were flushed with CO_2 -free air for 3 minutes, lowering the CO_2 concentration in the chamber to $<5 \mu\text{L L}^{-1}$. The gas chamber was then sealed and incubated for 3 h. At the end of the incubation period 24 mL of gas was sampled from the chamber; 14 mL of which was injected into an infrared gas analyser (EGM-4, PP-Systems, Amesbury, USA) to measure the CO_2 concentration. The analyser was calibrated against a reference gas, BOC 450 $\mu\text{L L}^{-1}$ carbon dioxide certified gas. The remaining 10 mL was injected into an N_2 flush-filled 12 mL gas vial (Labco) and analysed for $^{13}\text{C}\text{-CO}_2$. The $^{12}\text{C}/^{13}\text{C}$ ratio of each gas sample was measured using a gas bench (Delta^{plus} Advantage Thermo Scientific, Bremen, Germany) interfaced with an isotope ratio mass spectrometer (Trace Ultra GC Thermo Scientific, Bremen, Germany). Prior to sample injections, a reference gas (IAEA reference material NBS 19 TS-Limestone) was pulsed through the unit three times; the third pulse was used to calibrate the instrument. Using a quality controlled standard, the precision of the instrument was recorded as $\delta^{13}\text{CO}_2$ 0.24‰ (\pm SD of the mean).

219 **Isotopic mass-balance partitioning**

220 To partition the CO₂ efflux into its root- and SOM-derived components a mass balance
 221 model was used (equation 2) where the end-members were the $\delta^{13}\text{C}$ of the root respiration
 222 and the respiration derived from the respective unplanted treatments (control and nutrient
 223 amendment treatments). To obtain the $\delta^{13}\text{C}$ of the root respiration (as a best proxy for root-
 224 derived respiration which would include microbial mineralisation of root-derived
 225 substrates), immediately after destructively harvesting the root material, approximately 0.3
 226 g of root was placed in a vial. The vial was flushed with CO₂-free air at a flow rate of 150 mL
 227 min⁻¹ for several minutes and then incubated for 30 minutes at room temperature. The
 228 headspace was then transferred to another vial by peristaltic pump. The $\delta^{13}\text{C}$ signature of
 229 the CO₂ was determined as above (Midwood et al., 2006).

$$230 \quad P_{\text{root respiration}} = (\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_0) / (\delta^{13}\text{C}_{\text{root respiration}} - \delta^{13}\text{C}_0) \quad (\text{equation 2})$$

231 Where:

232 P_{root} = proportion of the total CO₂ that was root-derived

233 $\delta^{13}\text{C}_{\text{sample}}$ = $\delta^{13}\text{C}$ signature of the sample obtained at each measuring point

234 $\delta^{13}\text{C}_0$ = average $\delta^{13}\text{C}$ signature of the control treatment

235 $\delta^{13}\text{C}_{\text{root respiration}}$ = $\delta^{13}\text{C}$ value of root respiration

236 On Day 57 all pots were destructively harvested. Shoots were clipped to the soil surface.
 237 Each plant was split into root and shoots. The soil was removed from the pot and roots
 238 carefully removed by hand; the loose soil (bulk soil) was bagged and stored at 4°C. The soil
 239 attached to root (rhizosphere soil) was collected by washing roots 3 times with deionised
 240 water, this soil slurry was then freeze-dried. After washing, roots were stored at -20°C until

freeze-dried. Once freeze-dried, root and shoots were weighed, ball-milled and analysed for total N, total C (Flash 200 elemental analyser, Thermo scientific, Bremen, Germany), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Flash EA 112 series coupled to a Thermo Finnegan delta plus Xp, Bremen, Germany).

Microbial biomass

For each pot, two aliquots of soil (12.5 g dry weight) were taken. One aliquot was extracted immediately with 0.5 M K_2SO_4 . The extractant was shaken for 30 min and filtered through Whatman no. 42 filter paper. The second aliquot was fumigated with chloroform for 24 h and extracted as above (Vance et al., 1987). Both aliquots were analysed for total organic carbon (TOC-500A; Shimadzu, Kyoto, Japan). Microbial biomass size was calculated by taking the TOC of the non-fumigated from the fumigated and dividing by the K_{ec} factor (0.45) (Joergensen, 1996).

Soil mineral N

An aliquot of soil (20 g fresh weight) was extracted with 100 ml of 2 M KCl. Samples were shaken for 1 h and stored at 4°C. Samples were analysed for NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ on a Skalar, continuous flow analysis (FIA- Skalar, Breda, the Netherlands) within 3 days of extraction.

Statistical analyses

Statistics on these data were carried out using Genstat 15th Edition (VSN international, Hemel Hempstead, UK). Data were log transformed when the data did not follow normal distribution. General analysis of variance (ANOVA) was used to determine significance of soil type, treatment and time on total CO_2 respiration, SOM-derived respiration and root-derived respiration. ANOVA was used to determine the significance of soil type on microbial

264 biomass C and significance of treatments on total plant biomass, plant N uptake and root-
265 to-shoot ratio. Where effects were significant ($P \leq 0.05$), the significance of differences
266 between individual means was determined by least significant difference (LSD).

267

Results

Total CO₂ respiration

In both soils, presence of plants significantly increased ($P < 0.001$) total CO₂ efflux. For the high productivity soil a 67% and 87% increase in total CO₂ respiration, over the experimental period, was observed for the planted and planted with nutrient addition treatments relative to the corresponding unplanted treatments, respectively. Likewise, for the low productivity soil a 50% and 87% increase in total CO₂ respiration was observed for the planted and planted nutrient addition treatment relative to the corresponding unplanted treatments, respectively. Soil type significantly ($P < 0.001$) affected basal SOM mineralisation in unplanted soils, with soil CO₂ efflux from the unplanted high productivity soil approximately double that of the low productivity soil. Rates of basal SOM mineralisation were unaffected by nutrient addition for either soil type, and did not change significantly over the course of the experiment.

SOM-derived respiration

For the high productivity soil, a significant ($P < 0.001$) increase in SOM-C mineralisation was observed in the planted treatment not receiving nutrient additions relative to the unamended, unplanted soil (positive priming) on days 21 and 28. For the low productivity soil, no significant difference in SOM-C mineralisation was found between the planted and unplanted treatment not receiving nutrient additions (Figure 1). For both soils, on day 21, a significant influence ($P < 0.001$) of nutrient additions on SOM-derived CO₂ efflux was observed in planted treatments. Nutrient addition resulted in a significant reduction ($P < 0.001$) in SOM-C mineralisation in the planted treatments for days 35-56 in the high productivity soil and on day 56 in the low productivity soil relative to SOM-C mineralisation of the unamended treatment of the high and low productivity soil, respectively. Soil type

significantly affected ($P < 0.001$) SOM-C mineralisation in all treatments, with higher mineralisation in the high productivity soil (Figure 1).

Clipping was found to affect rates of SOM-C mineralisation measured 24 h after removal of leaf tissue, with the magnitude and direction of changes were dependent on soil nutrient treatments (Figure 3 and 4). Effects of clipping were most consistent over time in soils receiving nutrient additions. For the high productivity soil, clipping reduced SOM-C mineralisation ($P < 0.001$) on day 22, but SOM-C mineralisation was significantly increased on days 36, 50 and 57 (Figure 3B). Similarly, for the nutrient amended low productivity soil, clipping significantly increased ($P < 0.001$) SOM mineralisation on days 22, 50 and 57 (Figure 4B).

Root-derived respiration

A continuous increase in root-derived respiration was observed throughout the experiment for all treatments except for the low productivity, unfertilized soil. For this treatment, root-derived respiration was found not to change significantly over time (Figure 2). The planted, nutrient amended, high productivity soil treatment had the greatest rate of root-derived CO₂ respiration (Figure 2). Nutrient addition supported increased root-derived respiration in both soils. For the high productivity soil, without nutrient addition, clipping significantly decreased ($P < 0.001$) root derived respiration 24 h after clipping on Day 22 (Figure 3C). Clipping reduced ($P < 0.001$) root-derived respiration in both soils when they add received nutrient amendment (Figure 3D, 4D). For the low productivity soil, without nutrient addition, clipping significantly increased ($P < 0.001$) root-derived respiration 24 h after clipping on Day 57 (Figure 4C).

314

315 Plant biomass

316 Soil type significantly ($P < 0.001$) affected total plant biomass and N-uptake with high
317 productivity soil supporting greater total plant biomass and N uptake (Table 1). Root
318 biomass was greater for high productivity soil ($P < 0.001$) compared to the low productivity
319 soil (Table 1) for both plant treatment and plant & nutrient treatment. In both soils, addition
320 of nutrients significantly ($P < 0.001$) increased total shoot biomass but did not significantly
321 affect total root biomass. Addition of nutrients significantly ($P < 0.001$) reduced the shoot-
322 to- root ratio in both soils.

323

324 Discussion

325 In support of our first hypothesis, application of nutrients reduced SOM mineralisation in
326 planted soils, particularly later in the experimental period (> 42 d plant growth, Figure 1).
327 This effect was evident for both the high and a low productivity grassland soil, but was
328 larger in the high productivity soil. Our results indicate that this was specifically an effect of
329 nutrient availability on the plant-mediated component of SOM mineralisation (i.e. negative
330 priming, Kuzyakov, 2010), as nutrient additions to unplanted soils did not change basal SOM
331 mineralisation rates.

332 Priming effects

333 Continuous ^{13}C -labelling allowed quantitative partitioning of CO_2 efflux into plant- and SOM-
334 derived components, and plant-mediated effects on SOM mineralisation were determined
335 by reference to rates from unplanted treatments (Thornton et al., 2004; Paterson et al.,

2008). This approach allowed us to establish that in the absence of nutrient additions root inputs increased SOM mineralisation in the high productivity soil only, and that nutrient additions reduced SOM mineralisation in both soils when planted (Figure 1). Therefore, in the high productivity soil, nutrient addition resulted in a switch from positive plant-mediated priming of SOM mineralisation to a reduction of SOM mineralisation rate relative to unplanted soil (i.e. negative priming Blagodatskaya et al., 2007). For the low productivity soil, the nutrient addition effect on SOM mineralisation was less, but also resulted in a significant negative priming effect of plants by the end of the experimental period (Figure 1).

For both soils the nutrient additions did not have direct effects on the basal rates of SOM mineralisation, or on the size of the microbial biomass (Table 1), suggesting that nutrient additions did not impact microbial processes mediating SOM mineralisation directly, and that impacts were a consequence of modified interactions between roots and microbial communities (discussed below). This is evidence that the controls of basal and primed components of SOM mineralisation (and N_M) are distinct, as has been suggested previously (Paterson, 2009; Murphy et al., 2015). The insensitivity of basal SOM mineralisation to nutrient addition is consistent with available C-substrate being the primary limitation to microbial activity in soil (Schimel and Bennett, 2004). It is notable that this was the case for both soils, despite their differing N-supply capacities for plant growth under field conditions (McDonald et al., 2014), and evident here in the increased biomass production supported by the high productivity soil (Table 1). This highlights that the plant and microbial components interacting in these soils were subject to differing constraints which, as we will now discuss, may be fundamental to how root-soil interactions can variably affect N_M .

The magnitude of priming effects has been found to be related to the rate of labile substrate input into soils (Paterson and Sim, 2013). Consequently, reduced relative allocation of plant assimilate below-ground in response to increased nutrient availability (Merckx et al., 1987; Liljeroth et al., 1990) may function to lower priming of SOM mineralisation directly. However, in this experiment although we found significant reductions in root-to-shoot ratio as a consequence of nutrient additions to both soils (Table 1), this was a result of increased shoot production, with root biomass not being significantly affected (at final harvest on day 57, Table 1). In addition, the root-derived CO₂ efflux, of which microbial mineralisation of rhizodeposits is a component, was increased in nutrient-amended treatments. In combination, these results are not consistent with reduced rhizodeposition being the cause of negative priming in the soils receiving nutrient additions. Further, from 21 days into the plant growth period the SOM mineralisation rate was reduced such that it was less than that of basal mineralisation in unplanted soils, meaning that the changed rate cannot be explained simply as a reduction in plant-mediated SOM mineralisation. Such negative priming effects of plants have been reported previously (Kuzyakov and Bol, 2006; Blagodatskaya et al., 2007), and have been interpreted as resulting from preferential microbial use of rhizodeposits over substrates derived from SOM (Blagodatskaya and Kuzyakov, 2008). That this preferential use of rhizodeposits was not a dominating process in the absence of nutrient additions suggests that SOM is utilised as a nutrient source by microbial communities in the rhizosphere, and that this process is accelerated (primed) when their prevailing C-limitation is alleviated by rhizodeposition (Paterson, 2009).

This view of priming as a microbial nutrient-mining process (Craine et al., 2007) is supported by evidence that under N-limited conditions priming mobilises N-rich components of SOM

(Murphy et al., 2015), and this has significant implications for plant regulation of soil nutrient cycling. Root interactions with the rhizosphere microbiome have been considered as 'indirect symbioses' (Cheng et al., 2014), where rhizodeposition provides substrates to support microbial populations able to mineralise SOM nutrient sources. However, in their recent review Cheng et al. (2014) highlighted that if such interactions are co-evolved mutualistic strategies between plant and microbial partners then, unlike intimate rhizobial or mycorrhizal associations, they may be subject to cheating strategies where microbial populations not active in SOM mineralisation may still benefit from C-flow from roots. Further, through application of a model applying principles of game theory to account for cheating strategies, it was concluded that although stable mutualistic associations were possible, these were not an inevitable outcome of plant-microbe co-evolution (Cheng et al., 2014). However, our data are consistent with the view that priming effects are regulated by the factors limiting microbial activity, i.e. SOM mineralisation is promoted under conditions where the community has access to labile C and is limited by nutrient availability. Therefore, if the community closely associated with roots is limited by the availability of nutrients, then as a whole that community would shift activity toward acquisition of the resource(s) most limiting to it (Sinsabaugh et al., 2008). From a plant-centric perspective, this represents mobilisation of nutrients from soil pools with half-lives potentially much longer than plant roots, into pools that are turning over much more rapidly (Raynaud et al., 2006), and in doing so cycling nutrients into plant-available forms. While it is certainly possible that at a fine scale, plants manipulate their associated microbiome (Chapman et al., 2006), for example in recruiting a community including members capable of nutrient mobilisation, the mutualistic association would not be hijacked by 'cheating' populations, if the function of that community is regulated by its gross stoichiometric requirements.

In the high productivity soil we found that plant-microbe interactions mediating SOM mineralisation were uncoupled when nutrients were applied, consistent with microbial communities switching from use of SOM as a nutrient source to use of readily available mineral forms. However, this negative priming effect was reduced in this treatment immediately following clipping of shoot material (Figure 3 & 4). The periodic clipping, which was necessary to maintain the *L. perenne* plants in a manageable condition, resulted in a transient lowering in root-derived CO₂ efflux (Figure 3 & 4). The exact mechanisms underpinning this close temporal coupling of plant and microbial processes in the fertilised high productivity soil are unclear, not least because lowered root respiration (Kuzakov and Cheng, 2001) and lowered rhizodeposition may each contribute to the impact on root-derived CO₂ efflux. However, in the context of reduced negative priming, we suggest that the result is consistent with clipping lowering the quantity of rhizodeposits and the potential for microbial communities to preferentially utilise this C-source over that derived from the basal mineralisation of SOM.

Soil-specific influence

For the nutrient addition treatments, plants acquired equivalent amounts of the ¹⁵N applied in nutrient amendments from both soils (Table 1). Therefore, the differential capacities of the soils to supply N were defined by their distinct rates of N_M. For the high productivity soil, the larger N_M was both from a larger basal rate of SOM mineralisation (supporting a larger microbial biomass size in unplanted soil, (Table 1), and from a significant, positive plant-mediated priming effect in the unfertilised treatment (Figure 1). The lesser mineralisation rate and absence of significant priming in the low productivity soil suggest that SOM in this soil is relatively more protected from microbial decomposition compared to the high productivity soil, and that this underpins the low productivity soil's lesser capacity to

support plant growth. However, the relative importance of recalcitrance (Kleber, 2010), occlusion and organo-mineral stabilisation (Lützow et al., 2006) as limitations to basal and primed SOM mineralisation rates remain unclear. We suggest that to advance this understanding it will be necessary to study these mechanisms in systems where feedbacks between plant growth and soil processes can be maintained, particularly as our results indicate that rhizodeposition and nutrient availability modify the soil processes intrinsic to these interactions significantly. Whether plants are actively involved in down-regulation of priming under transient nutrient replete conditions, or whether ecotypes adapted to environments contrasting in nutrient cycling rates exhibit differing strategies to modify microbial processes also remains unclear (Chapman et al., 2006)). However, as the bulk of exudation from living roots is in the form of passively released, chemically labile, high C-to-N compounds that can be utilised very widely within microbial communities (Blagodatskaya and Kuzyakov, 2008), we suggest that such plant-directed control is not required for the indirect mutualistic association to function.

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606 **Figure Captions**

607 Figure 1: Soil organic matter mineralisation in two contrasting soils (high and low
608 productivity) measured as CO₂-C efflux ($\mu\text{g C g}^{-1} \text{ soil h}^{-1}$) from days 21-56 of incubation
609 period. Circles indicate high productivity soil, triangles indicate low productivity soil. Solid
610 lines indicate planted treatment and dotted lines indicate plant & nutrient treatment. Error
611 bars represent $\pm 1 \text{ s.e.m}$ (n=4).

612 Figure 2: Root-derived respiration in two contrasting soils (high and low productivity)
613 measured as CO₂-C efflux ($\mu\text{g C g}^{-1} \text{ soil h}^{-1}$) from days 21-56 of incubation period. Circles
614 indicate high productivity soil, triangles indicate low productivity soil. Solid lines indicate
615 plant treatment and dotted lines indicate plant & nutrient treatment. Error bars represent \pm
616 1 s.e.m (n=4).

617 Figure 3: Clipping effect for high productivity soil, 3A) SOM- derived CO₂ for planted
618 treatment 3B) SOM- derived CO₂ for plant & nutrient treatment 3C) root- derived
619 respiration plant treatment 3D) root- derived respiration plant & nutrient treatment.
620 Measured as CO₂-C efflux ($\mu\text{g C g}^{-1} \text{ soil h}^{-1}$) from days 21-57 of incubation period. Stars
621 indicate significant difference ($P < 0.001$). Error bars represent $\pm 1 \text{ s.e.m}$ (n=4).

622 Figure 4: Clipping effect for low productivity soil, 4A) SOM- derived CO₂ for planted
623 treatment 4B) SOM- derived CO₂ for plant & nutrient treatment and root derived respiration
624 4C) root- derived respiration plant treatment 4D) root- derived respiration plant & nutrient
625 treatment. Measured as CO₂-C efflux ($\mu\text{g C g}^{-1} \text{ soil h}^{-1}$) from days 21-57 of incubation period.
626 Stars indicate significant difference ($P < 0.001$). Error bars represent $\pm 1 \text{ s.e.m}$ (n=4).

| Parameter/Treatment | soil type | Control | nutrient | planted | planted & nutrient |
|--|-----------|--------------|--------------|--------------|--------------------|
| Microbial Biomass size ($\mu\text{g C g}^{-1}$ dry soil) | HPS | 598.2 (19.2) | 642.5 (28.8) | 653.3 (24.5) | 618.4 (69.2) |
| | LPS | 293.7 (18.1) | 254.5 (21.6) | 261.0 (12.4) | 351.8 (78.2) |
| Total plant biomass (g dry weight) | HPS | - | - | 4.5 (0.3) | 6.8 (0.2) |
| | LPS | - | - | 2.6 (0.1) | 5.0 (0.0) |
| Total N uptake (mg dry weight) | HPS | - | - | 124.4 (2.4) | 274.3 (3.8) |
| | LPS | - | - | 73.2 (2.5) | 215.1 (1.5) |
| Fertilizer N uptake (mg dry weight) | HPS | - | - | - | 123.45 (5.13) |
| | LPS | - | - | - | 125.27 (1.17) |
| Root biomass (g dry weight) | HPS | - | - | 1.42 (0.25) | 1.24 (0.20) |
| | LPS | - | - | 0.61 (0.07) | 0.62 (0.03) |
| Shoot biomass (g pot^{-1}) | HPS | - | - | 3.034 (0.04) | 5.5 (0.02) |
| | LPS | - | - | 1.99 (0.02) | 4.39 (0.04) |
| Total CO₂ efflux ($\mu\text{g g}^{-1}$ dry soil) | HPS | 50.31 (2.50) | 50.87 (1.66) | 79.38 (5.60) | 93.98 (3.40) |
| | LPS | 24.98 (0.53) | 25.42 (2.56) | 37.12 (2.82) | 47.04 (4.03) |
| Post incubation NH₄ (mg NH ₄ -N kg^{-1} dry soil) | HPS | 3.3 (0.2) | 1.2 (0.1) | 2.3 (0.2) | 0.3 (0.1) |
| | LPS | 1.8 (0.3) | 0.9 (0.1) | 1.4 (0.1) | 1.9 (0.2) |
| Post incubation NO₃ (mg NO ₃ -N kg^{-1} dry soil) | HPS | 124.3 (1.5) | 219.3 (7.1) | 0.7 (0.3) | 1.0 (0.3) |
| | LPS | 57.1 (1.8) | 154.7 (3.2) | 1.0 (0.3) | 11.1 (5.0) |

Fig 1







